ISSN 0975-2366
DOI:https://doi.org/10.31838/ijpr/2020.SP2.464

Research Article

Molecular detection of Mycobacterium tuberculosis by IS 6110 in patients with pulmonary tuberculosis in Babylon Province-Iraq

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Received: 04.07.20, Revised: 09.08.20, Accepted: 08.09.20

ABSTRACT
Insertion sequence (IS) 6110 is found at multiple sites in the Mycobacterium tuberculosis genome and displays a high degree of polymorphism with respect to copy number and insertion sites. Therefore, IS6110 is considered to be a useful molecular marker for diagnosis and strain typing of MTB. Tuberculosis, a communicable disease with significant morbidity and mortality, is the leading cause of the death in the world from bacterial infectious disease. Because of its public health importance, there is need for rapid and definitive method of detecting the causative organism. Several approaches have been attempted, but the molecular methods especially Polymerase Chain Reaction assays are the most promising for rapid detection of M. tuberculosis complex from clinical samples.

Keywords: Mycobacterium tuberculosis, Tuberculosis, polymerase chain reaction.

INTRODUCTION

Tuberculosis, second most common infectious disease spreading their wings. Roughly one-third of the world's population has been infected with M. tuberculosis with new infections occurring in about 1% of the population each year [(1).] As per WHO Global TB report 2013, about 8.6 million people develop TB and 1.3 million died from TB and (including 320000 deaths among HIV positive people).

India alone accounted for 2.0-2.5 million cases in 2010, thus contributing approximately 26% of all cases worldwide.

According to National Tuberculosis Control Programmes, 2.6 million new cases of sputum smear positive pulmonary tuberculosis; 2.0 million new cases of sputum smear negative pulmonary tuberculosis were observed in 2010 world wide [(2).] Early detection of tuberculosis by conventional method is time consuming because culturing can take 4-8 weeks. Direct staining and microscopy have low sensitivity and specificity and can provide only a preliminary diagnosis. PCR is the rapid detection technique with high sensitivity [(3).] PCR uses oligonucletide primers to direct the amplification of target nucleic acid sequences via repeated rounds of denaturation, primer annealing and primer extension. IS6110 is widely used target genes, due to the presence of its multiple copies in M. tuberculosis complex genome which is believed to confer higher sensitivity [(4).]

Mycobacteria are aerobic, non motile, acid fast bacilli, non spore forming bacteria. They are neither gram positive nor gram negative. Although they do not stain readily, once stained they resist decolorization by acid alcohol and retain carbolfuchsin stain, therefore called acid fast bacilli, the high lipid (waxy) content mycolic acid (approximately 60%) of their cell wall makes mycobacteria acid fast. The major human pathogen is Mycobacterium tuberculosis, the cause of both pulmonary and extrapulmonary tuberculosis [(5).]

MATERIALS AND METHODS

A maximum of 512 individuals were suspected of having tuberculosis after clinical symptoms and radiologic changes for tuberculosis were recorded for traditional laboratory diagnosis and were positive For at least one of the conditions for diagnosis. There were 15 patients diagnosed with either Zihel-Neelsen staining technique [(6)] or L.J.M culture [(7)] or both.

PCR for MTBC to detect the prescence or absence of IS6110 of MTB DNA using PCR master Mix containing (1U Taq DNA polymerase, 250μM dNTPs, 30mM KCL, 1.5mM MgCL2, 10Mm Tris-

HCL, 1.5μM Stabilizer), IS6110F(ctcgtccagcgccgcttcgg) and IS6110R (cctgcgagcgtaggcgtcgg) primers amplift a target fragment of 123bp. 20μL PCR reaction mixture containing (2.5μL of primers) and (5μL of DNA template, PCR preMix and Nuclease free water). The reactions were subjected to (Initiation denaturation)5min at 95°C, followed by 45 cycles each of 1min at 94°C(denaturation), 1min at 68°C (annealing) and 1min at 72°C (extension) with final extension of 10min at 72°C and 1min at 4°C (hold) [8].

Detection of amplified products

The amplified products were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. Agarose gel was prepared by dissolving 1.5% gm of agarose powder in 90 ml of 1X TBE buffer (pH = 8) in boiling water bath, allowed to cool to 50◦C and ethidium bromide at the concentration of 0.05 mg/ml was added.

The comb was fixed at one end of the tray for making wells used for loading DNA sample. The agarose was poured gently into the tray, and allowed to solidify at room temperature for 30 min.

The comb was then removed gently from the tray. The tray was fixed in an electrophoresis chamber filled with TBE buffer that covered the surface of the gel, 5μl of the amplified product was loaded into the wells in agarose gel and in one well we put the 5μl of DNA ladder. The electric current was allowed at 80 volts for 10 min.

RESULTS AND DISCUSSION

Out of the 512 suspected cases of confirmed pulmonary tuberculosis, there were 15 cases positive.

Sputum smear microscopy has been the primary method for diagnosis of pulmonary tuberculosis in low and middle income countries, it is simple, rapid and inexpensive technique which is highly specific in areas with a very high prevalence of TB, it also identifies the most infections patients and is widely applicable in various populations with different socio-economic levels [9].

This result true with a study performed showing that most of the smear positive are culture positive, but with treatment, it increases the chance of dead bacilli and smear positive can be culture negative in 98-100% cases. Mycobacterium culture is highly sensitive than AFB (the threshold for culture to be positive is 100 bacilli per ml of sample), but growth of TB bacilli on traditional solid medium requires 4-8 weeks and consequently delays appropriate treatment in the absence of a confirmed diagnosis [10].

Although the culturing of MTB was considered the gold standard test for diagnosis of MTB, it was low sensitivity and high specificity compared to AFB smear microscope because the culture of MTB require specific laboratory environment incubation reach to 8 weeks until getting colony on slant L-J medium which difficult with unstable electric power in laboratory setting, in addition to the sputum require processing to limit contaminants normal flora that lead to false positive/negative results [11].

All the 15 (100%) confirmed cases with pulmonary tuberculosis by AFB and culture were positive for the presence of IS6110 in the genome of M. tuberculosis by using the conventional PCR technique. Therfore IS6110 give high sensitivity, specificity, rapid hot spot for the diagnosis of tuberculosis.

The amplified product amplicon gives 123bp in gel electrophoresis as depicted in figure (1)

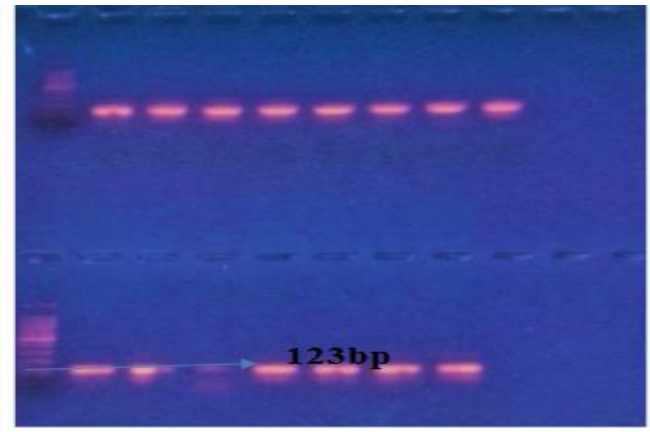

Fig.1: The amplified product amplicon gives 123bp in gel electrophoresis as depicted in figure

The conventional methods for laboratory diagnosis of M. tuberculosis include microscopy (acid fast staining) and culture for enhancing treatment strategies and reducing the potential of spreading TB in a community by pulmonary TB patients, new diagnosis methods for MTBC are needed to help combat this deadly disease, in which the use of nucleic acid amplification and detection in sputum, blood and body fluids may provide quick and specific results for identifying the MTBC [12].

PCR with its rapidity and higher sensitivity facilitates the early and accurate identification of the causative M. tuberculosis and thus very helpful for treatment, prevention and control of this chronic infectious disease.PCR as molecular diagnosis had been using for many years for the detection of Mycobacterial DNA. However, PCR of Mycobacterium DNA with one type of primer sometimes may cause ambiguity in the detection of MTB-DNA and therefore, should be checked with other primers specific for MTBC. Primer specific for the most conserved region IS6110 was used for the diagnosis of tuberculosis by PCR [13].

CONCLUSION

The study recommends that direct molecular detection of M. tuberculosis complex is sensitive and specific and polymerase chain reaction method should be used as an adjunct to other methods of laboratory diagnosis of tuberculosis, the rapidity and high sensitivity of PCR targeting IS6110 gene sequence compared to smear and culture, compensates for the higher cost of the test in the diagnosis of cases.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

CONFLICT OF INTEREST

None

FUNDING

Self-funding

REFERENCES

1. poongulali S, Sundararman M. Is tuberculosis difficult to diagnose in time? Status quo of modern diagnose and treatment. Biomedicine.2014;34(1):5-15.
2. Pingle P, Apte P, Trivedi R. Evaluation of microscopy, culture and PCR methods in the laboratory diagnosis of genitor- urinary tuberculosis. Am J Infect Dis Microbiol.2014;2(1):17-21.
3. Balakrishnan AS, Nandagopal B, Thangaraju K, Natarajan S. Comparative evaluation of two polymerase chain reactions targeting different genomic regions to detect Mycobacterium tuberculosis in sputum. Ind J Med Microbiol. 2010;28(4):303-07.
4. Jin XJ, Kim JM, Kim HK, Histopathology and TB-PCR kit analysis in differentiating the diagonsis of intestinal tuberculosis and Crohn's disease. World J Gastroenterol.2010;16:2496-503.
5. Alzubadi AAA. Interferon -gamma gene polymorphism as predisposing factor to pulmonary tuberculosis in Babylon Province-Iraq.2013.
6. Varaine F, Henkins M, Grouzard V. Tuberculosis: Practical guide for clinicians, nurses, laboratory technicians and medical auxiliaries.5th ed. Medicines Sans Frontieres-March.2010.
7. Brooks, Geo F., Karen C. Carroll, Janet S Butel, Stephen A. Morse, Jawetz, Melnick, Adelberg's Medical Microbiology, 25th Edition ,2010.
8. Abdul-Hussein ZM, Tarish HR, Aljanabi A. Molecular detection of M. tuberculosis in urinary Bladder FFPET samples could be a proposed specific Entity of Bladder Carcinoma.2015;5;2.
9. International Standards for tuberculosis Care. 2013.
10. Sarin, Singla R, Visalakshi P, Jaiswa A, Puri4 MM, Khalid UK, Mathuria K, Singla N, Behrra D, Sharma PP. Smear microscopy as surrogate for culture during follow up of pulmonary MDR-TB patients on DOTS plus treatment.2010.
11. Asmar S, Drancourt M. Chlorhexidine decontamination of sputum for culturing Mycobacterium tuberculosis. BMC microbiology. 2015,15(1):155.
12. Alli OAT, Ogbalu OD, Alaka OO. Direct molecular detection of MTBC from clinical samoles-An adjunct to cultural method of laboratory diagnosis of tuberculosis. N Am J Med Sci.2011;3(6):281-88.
13. Ashish K. Ashana, Molly Molly Madan. Study of Target gene IS6110 and MPB 64 in Diagnosis of Pulmonary Tuberculosis. International Journal of Current Microbiology and Applied Sciences.2015;4(8):856-63.